AMENDMENTS

Amended Specification:

Please amend the paragraph beginning on line 13, page 1 as follows:

Parenteral route of vaccination is primarily modeled after the demonstrated ability of BCG to alter the progression of Mycobacterium tuberculosis infection to its pulmonary disease state. The portal of infection is the lung. The site of primary infection is also the lung. What is poorly perceived is that pulmonary M. tuberculosis infection is due to hematogenous dissemination. Inhalation acquisition primarily involves the superior segment of the lower lobes or the right middle lobe. Gohn complexes occur in the lymphatic drainages of these sites of infection. Upper lobe disease is the consequence of bacillemia and metastatic implantation, which accounts for the success of IgG immunoglobulins and systemic T-cell immunity in precluding or arresting the development of metastatic disease in the upper lobes.

Please amend the paragraph beginning on line 1, page 2 as follows:

With Mycobacterium avium subspecies paratuberculosis (MAP), the portal of infection is the gastrointestinal tract. The target organ is also the gastrointestinal ~~disease~~ tract; however, gastrointestinal histopathology does not require a phase of systemic dissemination.

Please amend the paragraph beginning on line 13, page 2 as follows:

Drawing parallels between Mycobacterium tuberculosis and MAP, it is presumed that some cows with either Phase I and II infection may self-cure. Work done in conjunction with the Department of Pathobiology at the University of Florida, College of Veterinary Medicine has suggested that self-cure may occur in cows with Phase III disease. A cow with culture and serologically documented clinical Johne's disease was being serially bled ~~bleed~~ to obtain high titer sera. Prior to euthanasia, the animal ceased having diarrhea and began gaining weight. At necropsy, the gastrointestinal tract was basically normal. A rare histological section demonstrated MAP.

Please amend the paragraph beginning on line 14, page 3 as follows:

The present invention is also directed toward a method of generating a gastrointestinal, mucosally adherent, non-systemically invasive, live mucosal vaccine organism. The method of generating the vaccine organism includes selecting one or more mucosa-adherent organism strains based on desired binding affinity to an animal species' gastrointestinal tract; serially passaging the at least one strain in culture sufficient to significantly alter genomic expression; monitoring the passages for adherence to the mucosa of interest; demonstrating in-vivo alteration of the pathogenicity, including the mucosal histopathogenicity, and non-systemic invasiveness of the at least one passaged strain; and testing for the ability of the at least one passaged strain to confer protection against mucosal challenge by wild MAP strains.

Please amend the drawing description on line 7, page 5 as follows:

Figure 2 is a graph showing the relatively equivalent intestinal attachment of 5 different MAP strains within the gastrointestinal tract.

Please amend the methodology beginning on line 7, page 11 as follows:

MAP Strains: Five strains of MAP were used ~~in the pending publications~~: ATTC49164 (Strain A; Isolate from a patient with Crohn's disease), ATCC43015 (Strain B; Isolate from a patient with Crohn's disease), ATCC19698 (Strain C - isolate from a cow with naturally acquired paratuberculosis, MAP 728 (Strain E - a passage 10 of a clinical isolate) and MAP 7283 (Strain F - a passage 543 of a clinical isolate).

Please amend the methodology beginning on line 12, page 11 as follows:

Culture Methodology: A bacteriological loop was used to transfer the organisms to a 50ml cell culture flask containing 30ml of 7H9 broth with glycerol (Remel, Lenexa, KS). ~~S35~~ 35S Methionine (Trans35S-Label; ICN Biomedical, INC. Irvine, Ca) was added at a concentration of 8uCi per ml. All flasks were labeled appropriately and placed in a ~~37o C~~ 37° C, 5% CO2 incubator for three weeks.

Please amend the methodology beginning on line 13, page 12 as follows:

Attachment Procedure: The radiolabeled strains of MAP (strains A, B, C, E, and F) were scraped from each of the 50ml cell culture flasks, concentrated by centrifugation,

and the pellets washed three times with sterile saline. Pellets were then suspended in 15ml of RPMI-1640 with L-Glutamine (BioWhittaker, Walkersville, MD) and 10% fetal calf serum and sonicated for two minutes to break up clumps. After sonication, the organism were pushed through a 21 gauge needle to break up the remaining clumps and then the concentrated organisms were added to 110ml of RPMI with 10% fetal calf serum to total a total volume of 125ml. A sample of each strain of organism was taken and quantitative cultures done by serial dilution and plating on 7H11 agar (Remel, Lenexa, KS). An additional sample was taken to determine the disintegrations per minute (DPM) for the same size sample as that used for the quantitative cultures. This allowed us to determine the labeling efficiency for each strain of MAP. Twenty-five ml of RPMI solution containing the radiolabelled organisms was placed in each of the five petri dishes containing the different gut sections. This was done for all MAP strains in respectively labeled petri dishes with the gut sections. The petri dishes were then placed in a ~~37oC~~ 37° C, 5% CO2 incubator on an adjustable reciprocating orbital shaker for three hours. At the end of the incubation period, the strips of the intestine were washed three times with saline removing any unattached organisms. Three punches from each strip were taken from the center of each strip using a 6mm Miltex dermal biopsy punch to ensure that a constant size volume was taken and that edge effects were avoided. Punches were placed in labeled scintillation vials containing 3ml of Wallac Optisolv solubilizer and vials were placed in the ~~37oC~~ 37° C, 5% CO2 incubator overnight. Scintillation fluid (Wallac Optiphase Highsafe 3) was added to the scintillation vials and they were placed in scintillation racks for counting. The amounts of the organisms that bound to the intestine organ cultures were quantitated by scintillation counting to detect the 35S

methionine for 30 minutes per sample. DPMs per standard area were recorded and converted to CFUs by calculating the labeling efficiency of each strain of MAP on the day of the experiment and converting the DPM to CFU via multiplication of the ratio of CFU/DPM.

Please amend the figure legend on line 20, page 15 as follows:

> Figure 2 is a graph showing the relatively equivalent intestinal attachment of 5 different MAP strains within the gastrointestinal tract. There is no significant difference in the region of the intestine to which the organisms attached (P= .5214)